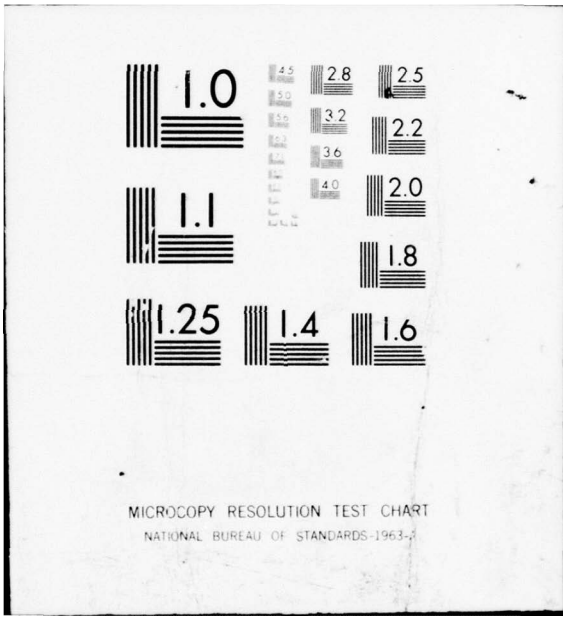
AD-A051 278 ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MASS F/G 6/1
HYPOTHERMIA FOLLOWING INJECTION OF 2-DEOXY-D-GLUCOSE INTO SELEC--ETC(U)
AUG 76 T SHIRAISH, M MAGER

UNCLASSIFIED USARIEM-M-5/7T NL

| OF |
AD
A051278

END
DATE
FILMED
4-78
DDC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Hypothermia Following Injection of 2-Deoxy-D-Glucose into Selected Hypothalamic Sites

Takemasa Shiraishi* and Milton Mager

U. S. Army Research Institute of Environmental Medicine

Natick, Massachusetts 01760

Current Address:

*The 1st Department of Physiology, School of Medicine

Tohkai University

Kanagawa, Japan

Running Title: Hypothermia Following 2-DG into Selected Hypothalamic Sites

Send Reprint Requests To:

Dr. Milton Mager
Director, Heat Research Division
U. S. Army Research Institute of Environmental Medicine
Natick, Massachusetts 01760

Abstract

From our previous studies with 2-deoxy-D-glucose (2-DG) an inhibitor of glucose utilization, we postulated that the resultant intracellular glucopenia affects central neuronal pathways involved in the control of peripheral heat production. In this investigation we have delineated these thermoregulatory sites by stereotaxically injecting micro-quantities of 2-DG into the hypothalamus of the rat and monitoring core temperature (T_{re}). After stabilization of T_{re}, 2 µl of 20 µg of 2-DG was injected into 350-400 g rats at 23 ± 1°C. Significant decreases in T_{re} were noted for the anterior hypothalamic, ventromedial and dorsomedial nuclei as well as the lateral and posterior hypothalamic areas. With the ventral premammillary nucleus (PMV) mean nadir T_{re} decreases of -1.1°C occurred 1 hour after administration of 2-DG, was significantly depressed after 3-1/2 hours, and returned to basal values after 4 hours. Dose dependant response was observed only for this nucleus. Of a total of 21 sites studied in both the anterior and posterior hypothalamus, the PMV, an area of unknown physiological function, was the most sensitive to glucose deprivation.

Key Words: hypothermia, 2-deoxy-D-glucose, hypothalamus, ventral premammillary nucleus, thermoregulation

Introduction

The administration of 2-deoxy-D-glucose (2-DG), a non-metabolizable analogue of glucose, to a variety of animals produces a marked intracellular glucopenia resulting from the competitive inhibition of phosphohexoseisomerase and consequently of glucose utilization. The lack of cellular glucose activates the sympathico-adrenomedullary system, and elicits a generalized hyperglycemia and hyperlipacidemia (3, 15, 33). Accompanying these alterations in circulating substrate are increased gastric acid secretion (5, 10, 22, 23), increased food intake (18, 25, 26), and decreased heat production (21). In addition, hypothermia has been noted in three of five patients administered 2-DG intravenously for cancer chemotherapy (14).

As a result of our studies with 2-DG in humans and mice (6), we have postulated that the disruption in glucose utilization affects central neuronal pathways involved in the control of peripheral heat production, and thus results in the observed reduction in core temperature. The present study presents a further characterization of the effects of 2-DG on thermoregulation. We have stereotaxically injected micro-quantities of 2-DG into selected sites of the hypothalamus of the rat and determined that the ventral premammillary nucleus is the site most sensitive to this analogue of glucose.

Materials and Methods

Approximately five hundred male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 350-400 g were used in the course of these studies. The animals were housed in individual cages with food (Charles River Rat Formula) and water ad lib, and kept in our animal holding room at least 14 days before being used. All experiments were performed at 23 ± 1°C, and before surgery a thermistor probe (YSI-401, Yellow Springs Instruments Co., Yellow Springs, OH) was inserted 4 cm to monitor core temperature (T_{re}). After baseline measurements were established, the animals were lightly anaesthetized with 25-30 mg/kg of i.p. pentobarbital sodium (Nembutal Abbott Laboratories, North Chicago, IL), and the stereotaxic surgery performed. After approximately 30 minutes, core temperature usually returned to baseline levels.

Fig. 1 illustrates the stereotaxic coordination method of Szentagothai et al. (27), which was used in all of our microinjection studies with the stereotaxic instrument (model No. 3-4, H. Neuman Co., Skokie, IL), and micro-manipulator (SM-4 Narishige, Tokyo). In this technique the vertical zero plane passes through bregma, and the horizontal zero plane lies 8 mm below the upper skull surface (Parietal bone). The microinjection experiments were acute preparations and carried out using a micropipette with an O. D. of 1.8 mm, I. D. of 1.0 mm and a tip diameter of 50 microns. The 2-DG solutions were always injected in a volume of 2 µl with a microsyringe (Roger Gilmont Instruments Co., Great Neck, NY), as was the physiological saline when used as a control. The 2-DG (Sigma Chemical Co., St. Louis, MO)

was dissolved in pyrogen-free physiological saline, and Methylene-Blue (Matheson, Coleman and Bell, Norwood, OH) was added in a concentration of 0.1% in order to aid histological identification of the injection site.

The microinjections with 2-DG were made into 21 different hypothalamic sites, and T_{re} monitored for a total of 4-8 hours. At the end of each experiment, the animal was injected with an overdose of pentobarbital sodium (60 mg/kg, i.p.), then perfused with saline and 10% formalin. The brain was removed, and fixed with the following solutions: (i) acid-alcohol formalin (40% formaldehyde 47.5 mℓ, acetic acid 47.5 mℓ, 95%, ethanol 450 mℓ and distilled water 405 mℓ): 48 hours, (ii) distilled water: 24 hours, (iii) sucrose-formalin (40% formaldehyde 100 mℓ, sucrose 300 g and distilled water 900 mℓ): 24 hours. Frozen coronal sections of 40-60 microns were cut on a microtome, mounted (gelatin 5 g, distilled water 500 mℓ, 80% ethanol 500 mℓ at 60°C) and stained using the Rucker-Koithan procedure (24). In Fig. 2 is illustrated the track of the micro-pipette and its tip after Nissl staining.

For monitoring anterior and posterior hypothalamic temperature, stainless steel cannulae were chronically implanted (L 1.0, V 9.0, P 1.0 and/or 3.7), under pentobarbital sodium anaesthesia, using the stereotaxic method previously described. The cannulae were made from sterile leur stub adaptors, 16 ga. (No. A1030 Clay Adams, Parsippany, NJ), and were fixed to the skull with dental cement. A week after surgery, a tissue implantation thermistor probe (YSI-520, Yellow Springs Instruments Co.) was inserted into

the permanent hypothalamic cannulae, while skin (tail) temperature was monitored by a YSI-409 and/or a YSI 44012 small surface thermistor. Oxygen consumption was measured on individual rats in an open system as previously described (21).

Results

In the left panel of Fig. 3 are plotted changes in the rectal temperature (mean ± S. E.) of three groups of 30 intact rats each administered 250, 375 or 562.5 mg/kg i.p. of 2-DG. Note the dose response to this analogue of glucose, and the resultant significant ($p<0.001$) regression line (right side of Fig. 3) for the change in T_{re} at 60 minutes after the administration of 2-DG. Although not depicted, saline controls did not exhibit significant changes in T_{re} from basal levels. In animals with implanted hypothalamic cannulae, similarly treated with 375 mg/kg of 2-DG, we observed depressions in the temperatures of both the anterior - and posterior hypothalamus, as well as decreased tail (skin) temperature (Fig. 4), and oxygen consumption (Fig. 5).

In Fig. 6 is depicted the effect on core temperature of the micro-injection of 20 μg/2 μℓ of 2-DG into selected hypothalamic areas. In the upper left hand corner are plotted the results with the anterior hypothalamic nucleus (NAH). Note that T_{re} decreased significantly ($p<.01$) after 15 minutes, exhibited a nadir of 1.6°C after 2 hours of the 2-DG administration, was still slightly but significantly depressed ($p<.05$) after 5.5 hours, but

back to baseline values by 6 hours. In the upper right hand corner are plotted the T_{re} changes noted with the dorsomedial nucleus (DM). In this area T_{re} was also significantly decreased after 15 minutes ($p<.025$), exhibited a nadir of 1.1°C at 2 hours, and was back to basal levels after 5.5 hours. In the middle left panel is depicted the T_{re} data for the ventromedial nucleus (VM). Significant T_{re} changes after 15 minutes ($p<.025$), maximum depression of 1.2°C at approximately 2 hours, a small but significant hypothermia ($p<.01$) after 5 and 5.5 hours, and normothermia at the end of the 6 hour observation period. In the middle right side figure is the data for the lateral hypothalamic area (AHL). At this site the response elicited with 2-DG is similar to that noted with NAH, i.e. significant depression after 15 minutes ($p<.025$) with a nadir after 1 1/2 - 2 hours of 1.5°C, and essentially back to basal values after 6 hours. In the bottom left panel are the results for the posterior hypothalamic area (AHP) the response of which is similar to that observed with DM in that after the initial significant depression at 15 minutes ($p<.025$), maximum fall at approximately 2 hours is only 1°C, with a slight but significant hypothermia over the last hour and a half. In the lower right hand panel are plotted T_{re} changes after microinjection into the medial mammillary nucleus (MM). Note that for portions of the observation period a slight hyperthermia was observed rather than the usual hypothermia.

The results obtained with the ventral premammillary nucleus (PMV) are plotted separately in Fig. 7, since this area was the most responsive to

2-DG administration, as reflected by the rapidity that nadir values in T_{re} were attained, the magnitude of the drop, and the rapid return to baseline values. Note that mean (n=9) T_{re} levels are significantly ($p<.01$) below basal values 15 minutes after the microinjection of 20 µg/2 µℓ of 2-DG, and that the nadir occurs after approximately 1 hour, and, while significantly depressed after 3 1/2 hours ($p<.01$), has returned to basal values after 4 hours.

In Table 1 are summarized the effects of 2-DG on body temperature when injected into 21 selected hypothalamic areas. The data presented in Fig. 6 and 7 are for 7 of these areas, and are representative of the responses of these 21 sites. Note that the T_{re} responses tabulated in Table 1 range from no change (-) as with the ML, to a moderate hypothermia (++) in the AHL, DMv and VMp, to an intense hypothermia (+++) in the PMV.

In Figure 8 are plotted the effect on core temperatures of different doses of 2-DG microinjected into the NAH and the AHL. Note that no dose response was obtained with either NAH with 40, 80 and 160 µg/2 µℓ of 2-DG, or with 20, 40 and 80 µg/2 µℓ injected into the AHL. In fact in the case of the latter area, the lowest dose evoked the greatest fall in T_{re}. On the other hand, 2-DG injected into the PMV not only resulted in a greater depression in T_{re} with 40, 80 and 160 µg/2 µℓ (Fig. 9), but also elicited significant ($p<.01$) dose-dependent responses.

Illustrated in Fig. 10 are a series of coronal rat brain sections which summarize the effects on body temperature of microinjected 2-DG into selected sites in the hypothalamus. In addition, these drawings are helpful

in visualizing the anatomical relationship and responses of the various areas we studied. Note that while specific sites in both the anterior and posterior hypothalamus reduced heat production following administration of 2-DG, maximum responsiveness (+++) was noted in the ventral premammillary nucleus (PMV); however, the adjacent dorsal premammillary (PMD), lateral mammillary (ML), prelateral mammillary (PL) and medial mammillary (MM) nuclei did not elicit a change in core temperature.

Discussion

Since 1885 it has been known that impulses flowing from peripheral thermoreceptors are integrated in the hypothalamus, and that the center controlling heat loss is situated in the anterior portion while the heat conservation center is located in the posterior hypothalamus (1, 11, 16, 28). Subsequently, many investigations have definitively demonstrated that mammalian temperature regulation is under hypothalamic control (2, 7, 8, 9, 31). Despite these and many other investigations, the role of the anterior hypothalamus in this thermoregulatory process is much better understood than the posterior area. In fact many investigators have considered the posterior hypothalamus as being "chemically blind" since no sensitivity to any chemical stimulation had been noted. Thus, in our investigations not only have we demonstrated that the posterior hypothalamus was responsive to micro-quantities of injected 2-DG, but in addition that the areas adjacent to the mammillary bodies, whose physiological function is even more poorly understood, also could respond to 2-DG with a generalized hypothermia.

Unquestionably, our knowledge of the anatomical connection of the mammillary bodies is greater than our understanding of their physiological function; thus, it is accepted that while its histological make-up is typical of limbic structures, which is atypical of the hypothalamus, functionally these bodies are considered part of the hypothalamus (13). Ranson, et al. (20) have demonstrated in a group of rhesus monkeys, that, following lesions made in an area dorsal and lateral to the mammillary bodies, several animals developed a pronounced hypothermia. Interestingly, the symptomatology and pathological findings in Wernicke's encephalopathy (4, 12, 19, 29, 30) are comparable, since individuals with this disease exhibit a significant hypothermia, and post mortem examination usually reveals marked damage to the posterior hypothalamus including the mammillary bodies. Indeed, a recent report (19) has documented a body temperature of 20.6^{o}C in one individual. Even more intriguing to us, is the clinical finding that many of the manifestations of this disorder, including the hypothermia, are ameliorated by treatment with thiamine. Since this vitamin is the prosthetic group of cocarboxylase, it is, of course, required if aerobic glycolysis is to proceed to completion. Thus, one can hypothesize that the hypothermia accompanying Wernicke's encephalopathy is a direct manifestation of neuronal lesions in the hypothalamus, which are well documented consequences of thiamine deficiency, or that the reduced core temperature is the result of incomplete glucose catabolism in the central nervous system.

The dose response curve obtained after i.p. administration of 2-DG to rats (Fig. 3), the depression in tail and hypothalamic temperatures (Fig. 4), and the precipitous decrease in oxygen consumption (Fig. 5) are consistent, with comparable data obtained with mice (21). In addition, the present observations also indicate a decrease in whole body heat production, rather than an increase in heat loss to the environment is responsible for the reduction in core temperature. The hypothalamic injections of micro-quantities of 2-DG (Fig. 6) also confirm our earlier hypothesis that the induced hypothermia with this glucose antagonist was predominantly the result of 2-DG effects in the central nervous system. This is also consistent with previous observations that brain tissue is more sensitive to the inhibitory effects of 2-DG than other tissues (32, 33). Additionally, Muller, et al. (17) have recently reported T_{re} depression with intraventricular injection of 2-DG of similar magnitude and time course as our data; however, they used 2.5 mg of 2-DG, while in our study only 20 µg was given.

It should be emphasized that of the 21 specific sites studied, areas in both the anterior and posterior hypothalamus were responsive to 2-DG as evidenced by significant decreases in body temperature; however, maximum sensitivity, as arbitrarily defined by the rapidity of the drop and return to normal, was noted with the ventral premammillary nucleus. This area not only evoked a precipitous decrease in core temperature, whose magnitude was dose dependent, but its return to basal values was also

comparatively rapid (4 hours). None of the other hypothalamic areas similarly challenged with 2-DG produced an equally rapid decline and recovery in body temperature as this nucleus. Also of interest is the observation that 2-DG administration into the nuclei immediately adjacent to the PMV, i.e. PMD, ML, PL and MM nuclei, did not elicit any alteration in core temperature. While generally little is known about the physiological function of the mammillary bodies, we can now ascribe a thermoregulatory involvement for one of the adjacent nuclei.

Acknowledgements

We wish to gratefully acknowledge the excellent technical assistance of SP-4 David Peiper and Mr. Theodore Mita.

References

1. Aronson, E. and J. Sachs. Die Berziehungen des Gehirns zur Korperwarme und zum Fieber. Pfluegers. Arch. Ges. Physiol. 37:232-301, 1885.
2. Bligh, J. Temperature regulation in mammals and other vertebrates. North-Holland Research Monographs, Frontiers of Biology , Vol. 30, North-Holland Publishing Co., Amsterdam, 1973.
3. Brown, J. Effects of 2-deoxyglucose on carbohydrate metabolism: Review of the literature and studies in the rat. Metabolism 11:1098-1112, 1962.
4. Campbell, A. C. P., and W. R. Russell. Wernicke's Encephalopathy: The clinical features their probable relationship to Vitamin B deficiency. Quart. J. Med. 10:41-64, 1941.
5. Colin-Jones, D. G. and R. L. Himsworth. The location of the chemoreceptor controlling gastric acid secretion during hypoglycemia. J. Physiol. 206:397-409, 1970.
6. Freinkel, N., B. Metzger, N. Harris, S. Robinson, and M. Mager. The hypothermia of hypoglycemia: Studies with 2-deoxy-D-glucose in normal humans and mice. New Engl. J. Med. 287:841-845, 1972.
7. Hardy, J. D. Physiology of temperature regulation. Physiol. Rev. 41: 521-606, 1961.
8. Hardy, J. D. Posterior hypothalamus and the regulation of body temperature. Fed. Proc. 32:1564-1571, 1973.
9. Hardy, J. D., A. P. Gagge, and J. A. J. Stolwyk (ed). Physiological and behavioral temperature regulation. Charles C. Thomas, Illinois, 1970.

10. Hirschowitz, B. I. and G. Sachs. Vagal gastric secretory stimulation by 2-deoxy-D-glucose. Am. J. Physiol. 209:452-460, 1965.

11. Isenschmid, R. and L. Krehl. Uber den Einfluss des Gehirns auf die Warmeregulation. Arch. exp. Path. Pharmak. 70:109-129, 1912.

12. Koppen, A. H., J. C. Daniels, and K. D. Barron. Subnormal body temperatures in Wernicke's encephalopathy. Arch Neurol. 21:493-498, 1969.

13. Krieckhaus, E. E. The mammillary bodies. Their function and anatomical connections. Acta. Biol. Exper. (Warsaw) 27:319-337, 1967.

14. Landau, B., J. Laszlo, and J. Stengle. Certain metabolic and pharmacologic effects in cancer patients given infusions of 2-deoxy-D-glucose. J. Natl. Cancer Instit. 21:485-494, 1958.

15. Landau, B. R. and H. A. Lubs. Animal response to 2-deoxy-D-glucose administration. Proc. Soc. Exp. Biol. Med. 99:124-127, 1958.

16. Meyer, H. H. Theorie des Fiebers und seine Behandlung. Zentbl. ges. inn. Med. 6:385-386, 1913.

17. Muller, E. E., A. Panerai, D. Cocchi, L. A. Frohman and P. Mantegazza. Central glucoprivation: Some physiological effects induced by the intraventricular administration of 2-deoxy-D-glucose. Experientia 29: 874-875, 1973.

18. Oomura, Y. Central mechanism of feeding. Advances in Biophysics (Tokyo) 5:65-142, 1973.

19. Philip, G. and J. F. Smith. Hypothermia and Wernicke's encephalopathy. Lancet II:122-124, 1973.

20. Ranson, S. W., C. Fisher, and W. R. Ingram. Hypothalamic regulation of temperature in the monkey. Arch. Neurol. Psychiat. 38:445-466, 1937.

21. Robinson, S. M., M. Mager and N. Freinkel. Interrelationship of central nervous system glucopenia and heat production in mice. The Pharmacology of Temperature Regulation. Ed. P. Lomax, E. Schonbaum, Switzerland, S. Karger, Basel., p. 112-123, 1973.

22. Shiraishi, T. Studies on the glucose-sensing cell (gastric type). J. Physiol. Soc. Jap. 32:203-218, 1970.

23. Shiraishi, T. and H. Takahashi. Relationship between glucose-sensing cell and gastric acid secretion. J. Physiol. Soc. Jap. 32:422, 1970.

24. Skinner, J. A. Neuroscience: Laboratory Manual., W. B. Saunders Co., Philadelphia, Pennsylvania, 1971.

25. Smith, G. P. and A. N. Epstein. Increased feeding in response to decreased glucose utilization in the rat and monkey. Am. J. Physiol. 217:1083-1087, 1969.

26. Smith, G. P. and A. W. Root. Effects of feeding on hormonal responses to 2-deoxy-D-glucose in conscious monkeys. Endocrinol. 85:963-966, 1969.

27. Szentagothai, J., B. Flerko, B. Mess, and B. Halasz. Hypothalamic Control of the Anterior Pituitary: An Experimental-Morphological Study. Akademiai Kiado, Budapest, 1962.

28. Thauer, R. Der Mechanismus der Warmeregulation. Ergebn. Physiol. 41: 607-805, 1939.

29. Thomsen, Z. Zur Pathologie und pathologischen Anatomie der acuten completen (alkoholischen) Augenmuskellahmung (Polioencephalitis acuta superior Wernike). Arch Psychiat Nervenks. 19:185-199, 1887.

30. Wernicke, C. Lehrbuch der Gehirkrrankheiten fur Arzte und Studierende, Berlin: Theodor Fisher, Vol. 2, p. 233, 1881.

31. Whittow, G. C. (ed). Comparative physiology of thermoregulation, Vol. II. Mammals. Acad. Press, New York, 1971.

32. Wick, A. N., D. R. Drury, H. I. Nakada, and J. B. Wolfe. Localization of the primary metabolic block produced by 2-deoxy-glucose. J. Biol. Chem. 224:963-969, 1957.

33. Woodward, G. E. and M. T. Hudson. The effect of 2-deoxy-D-glucose on glycolysis and respiration of tumor and normal tissues. Cancer Res. 14:599-605, 1954.

Figure Legend

Fig. 1. Stereotaxic coordination by method of Szentagothai et al. (27). Shown is a sagittal section of the rat's skull and brain to demonstrate the fixation plane of the head in the stereotaxic apparatus, and the orientation of vertical and horizontal reference planes. A: to anterior, P: to posterior.

Fig. 2. Microphotographs of the hypothalamus after Nissl staining. In A and B are the tracks of micropipettes, while in C, D and E are the tip of track of a same preparation at different magnification. The dark portion at the bottom of tip is the methylene blue dye. Magnification: C: x 35, B: x 50, A, D: x 70, E: x 100.

Fig. 3. Change in rectal temperature after intraperitoneal administration of 250, 375, and 562.5 mg/kg of 2-DG at 0 time. 30 different rats were used for each dose, and each point represents the mean ± S. E. In the figure at the right is the log dose-response curve for these experiments. Each point represents the mean depression in core temperature 60 minutes after the 2-DG injection. The straight line was drawn by the method of least squares, and is a highly significant ($p<0.001$) regression.

Fig. 4. Change in temperatures of the anterior hypothalamus (left panel), the posterior hypothalamus (right panel), rectal and skin (tail) after intraperitoneal administration of 375 mg/kg 2-DG. Each point represents mean ± S. E. of 10 (left panel) and 9 (right panel) rats.

Fig. 5. The effect of i.p. injection of 375 mg/kg of 2-DG on the oxygen consumption (ml/kg/min) of the rat. Each point depicts mean ± S. E. of 12 rats.

Fig. 6. Change in rectal temperatures after microinjection of 2-DG (20 µg/2 µl) into selected hypothalamic areas. Each point represents the mean of 6 to 9 rats ± S. E. NAH: anterior hypothalamic nucleus, DM: dorsomedial nucleus, VM: ventromedial nucleus, AHL: lateral hypothalamic area, AHP: posterior hypothalamic area, MM: medial mammillary nucleus.

Fig. 7. Change in rectal temperatures after 2-DG (20 µg/2 µl) injection into the ventral premammillary nucleus (PMV). Individual values for 9 rats are plotted on left portion, while the mean ± S. E. are in the right panel.

Fig. 8. Effects on rectal temperature of the microinjection of 40, 80 and *160 µg of 2-DG into the anterior hypothalamic nucleus (NAH) (upper graph)*, and 20, 40, 80 µg into the lateral hypothalamic area (AHL) (lower graph). 5 different rats were used for each dose, and each point represents the mean ± S. E.

Fig. 9. Dose-response of the microinjection of 40, 80, and 160 µg of 2-DG into the ventral premammillary nucleus (PMV). Each dose represents 6 different rats, and each point is the mean ± S. E.

Fig. 10. Summary of the effect on body temperature of the microinjection of 2-DG into selected hypothalamic areas, as depicted in a series of coronal rat brain sections. V LAT: lateral ventricle, Fx: fornix, CA: anterior commissure, APO: preoptic area, NAH: anterior hypothalamic nucleus, AHL: lateral hypothalamic area, SCH: suprachiasmatic nucleus, CHO: optic chiasma, SO: supraoptic nucleus, RCA: retrochiasmatic area, VMa: anterior subdivision of ventromedial nucleus, ARC: arcuate nucleus, DM: dorsomedial nucleus, VM: ventromedial nucleus, DMd: dorsal subdivision of dorsomedial nucleus, DMv: ventral subdivision of dorsomedial nucleus, VMp: posterior subdivision of ventromedial nucleus, AHP: posterior hypothalamic area, PMD: dorsal premammillary nucleus, PMV: ventral premammillary nucleus, PL: prelateral mammillary nucleus, MM: medial mammillary nucleus, ML: lateral mammillary nucleus. Refer to text (Legend Table 1) for explanation of + and - symbols.

Table 1. Summary of the effect on body temperature of the microinjection of 2-DG into 21 selected hypothalamic areas. Symbols used to denote the temperature response are as follows:

+++:	intense hypothermia	-:	no change (no hypothermia)
++:	moderate hypothermia	--:	slight hyperthermia
+:	hypothermia	?:	response not clear
±:	slight hypothermia		

"In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council."

"The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense."

(Fig)

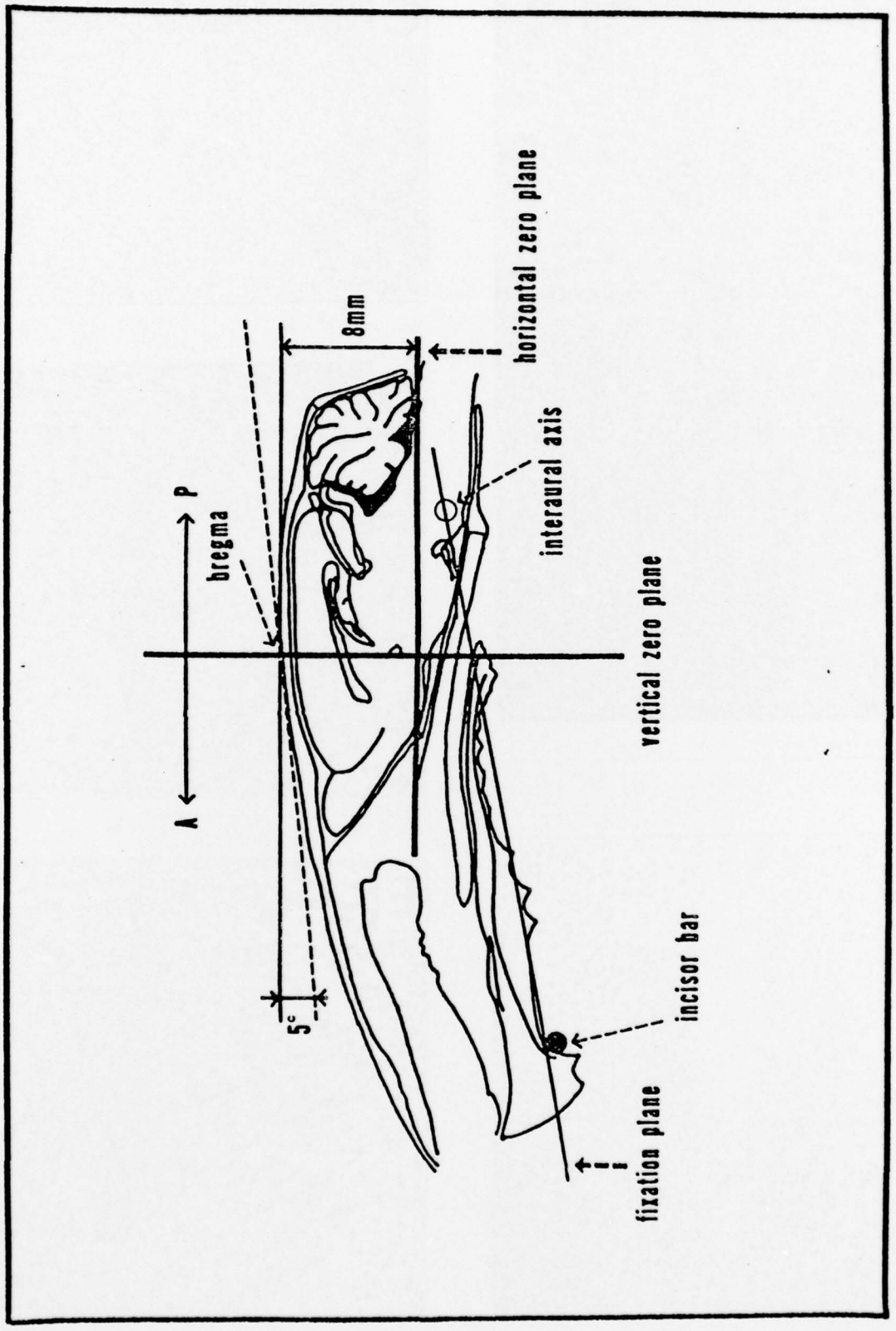
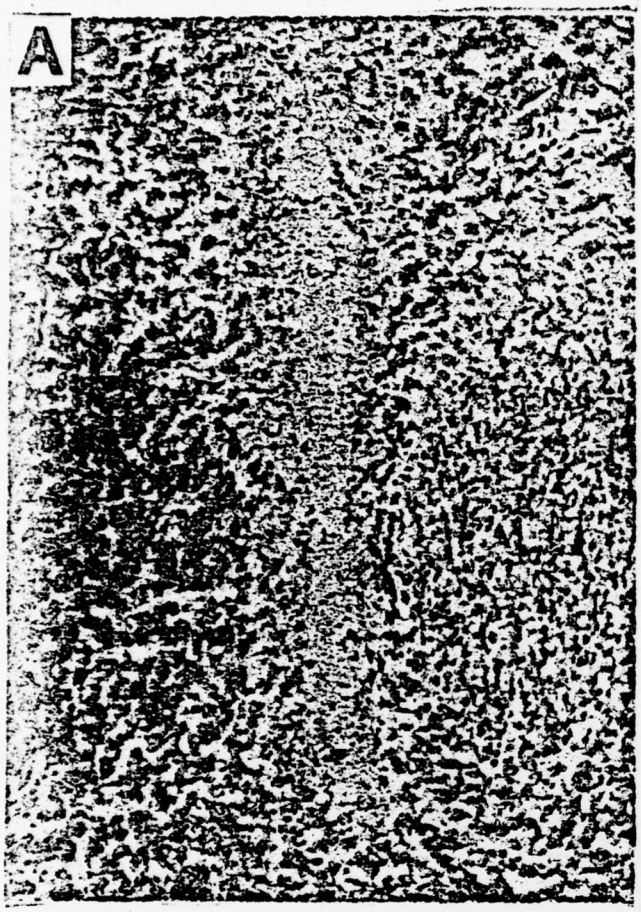
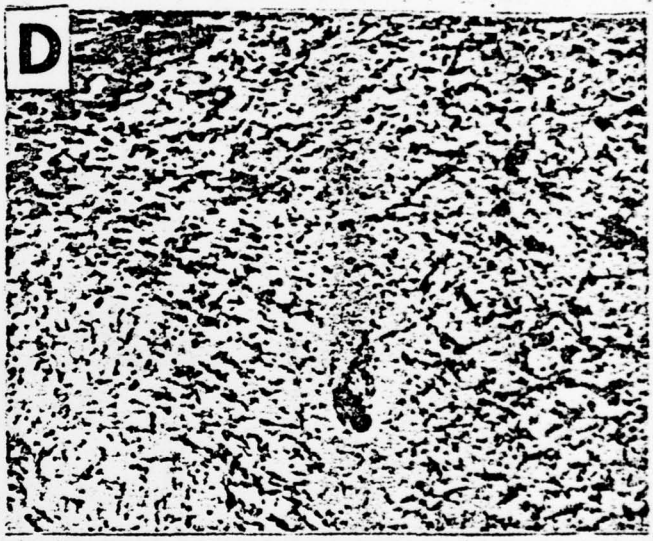
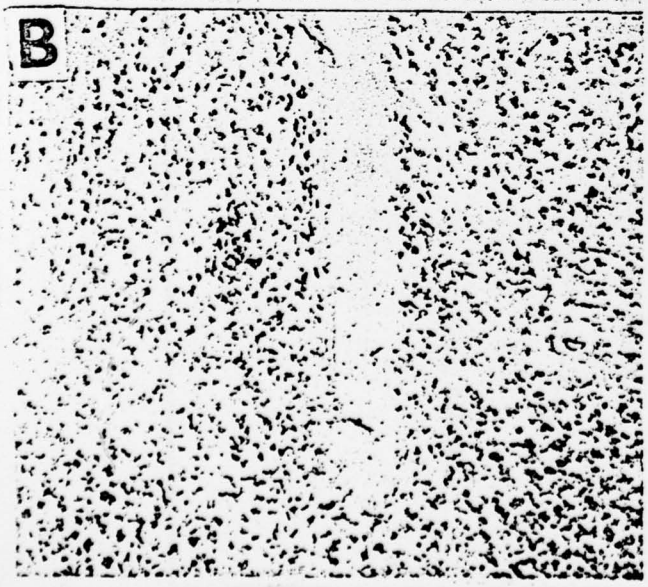
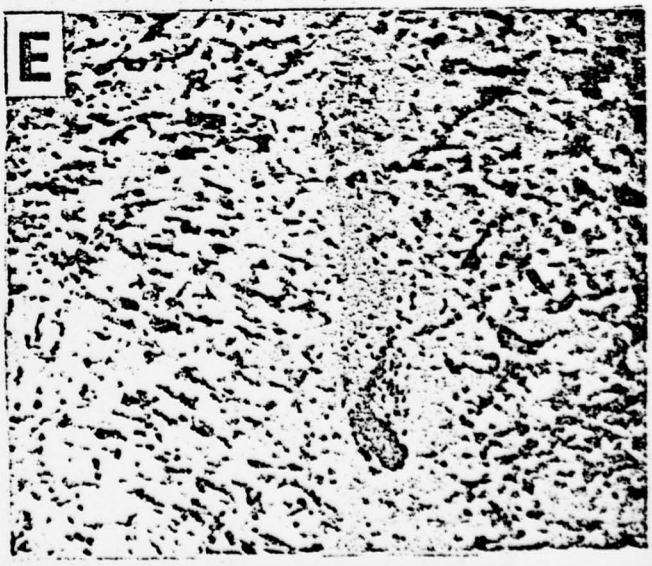

P
A
bregma
8mm
horizontal zero plane
interaural axis
vertical zero plane
5°
incisor bar
fixation plane

Fig 2









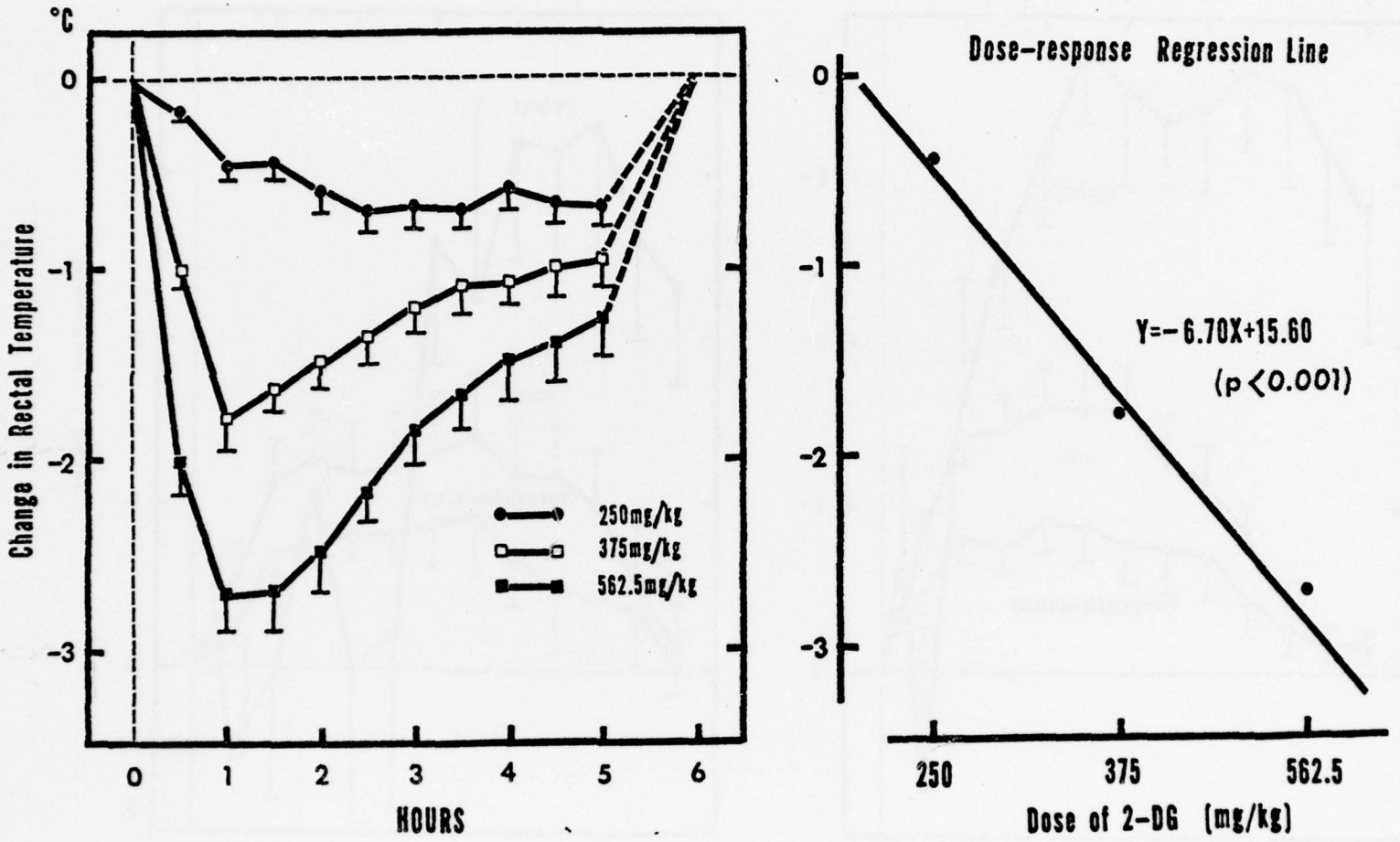

°C
0
-1
-2
-3
Change in Rectal Temperature
0
1
2
3
4
5
6
HOURS
250mg/kg
375mg/kg
562.5mg/kg
Dose-response Regression Line
Y=-6.70X+15.60
(p<0.001)
250
375
562.5
Dose of 2-DG (mg/kg)

Fig 3

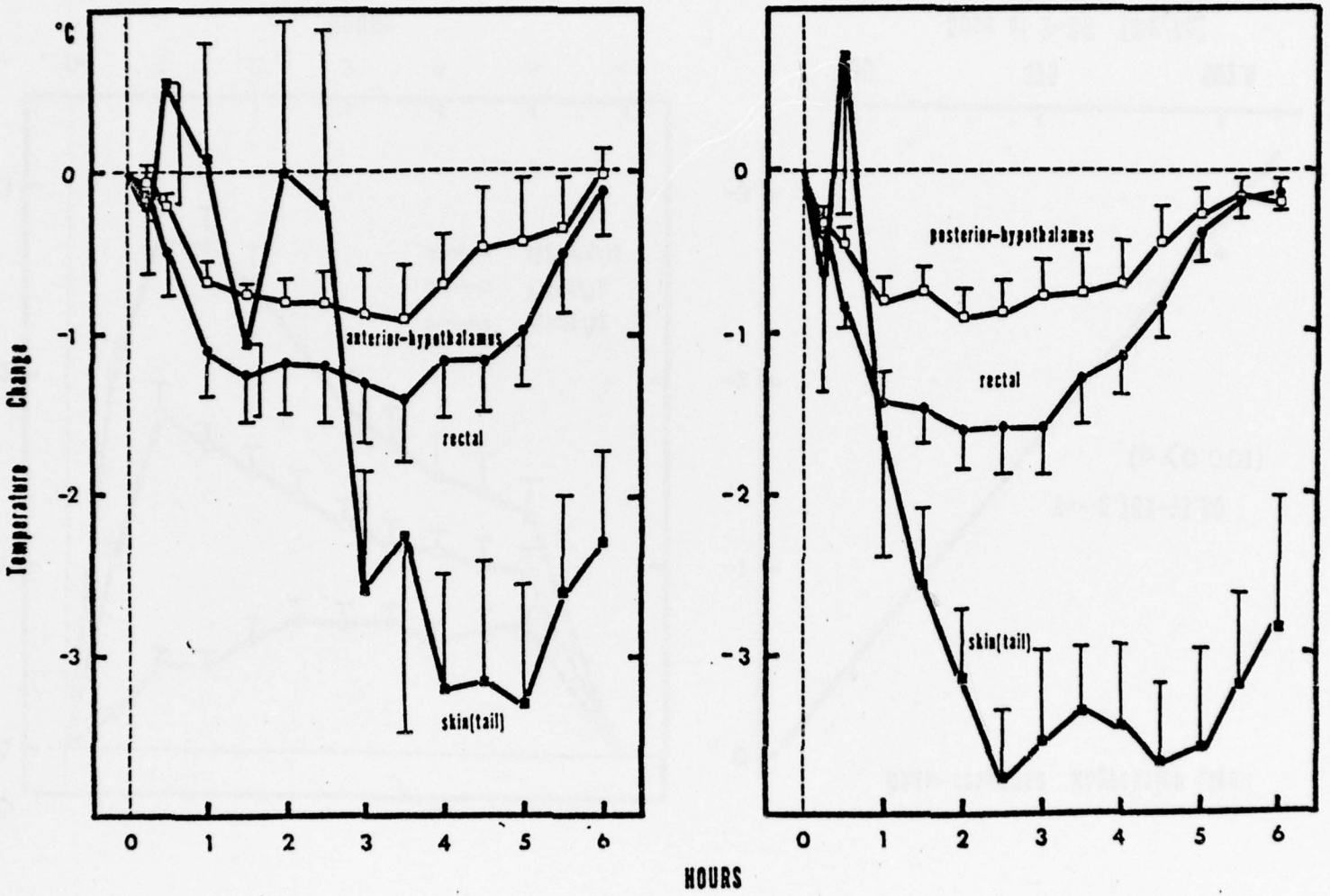

°C
Temperature Change
0
-1
-2
-3
anterior-hypothalamus
rectal
skin(tail)
posterior-hypothalamus
rectal
skin(tail)
0 1 2 3 4 5 6
HOURS

Fig. 4

Fig. 5

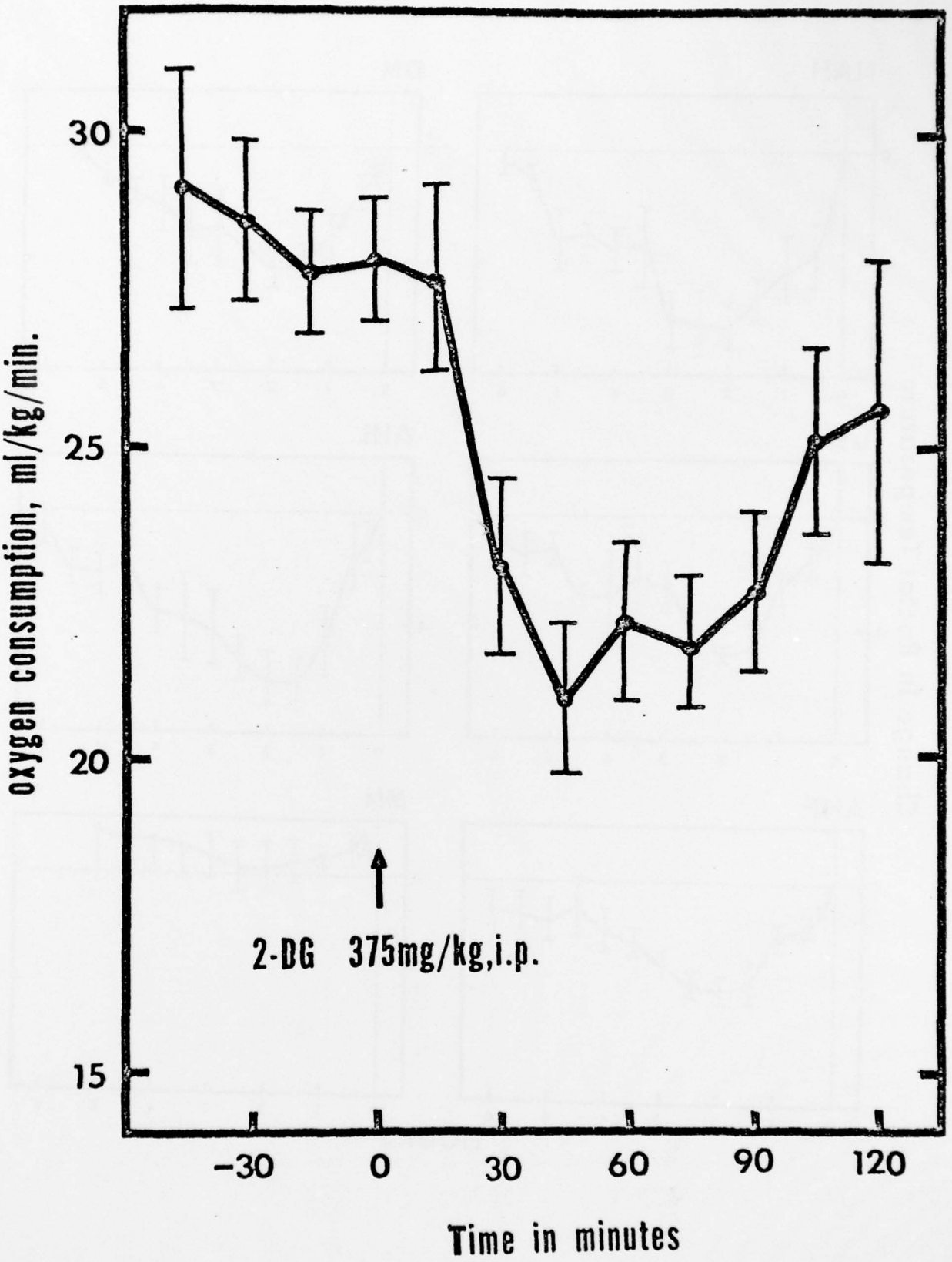
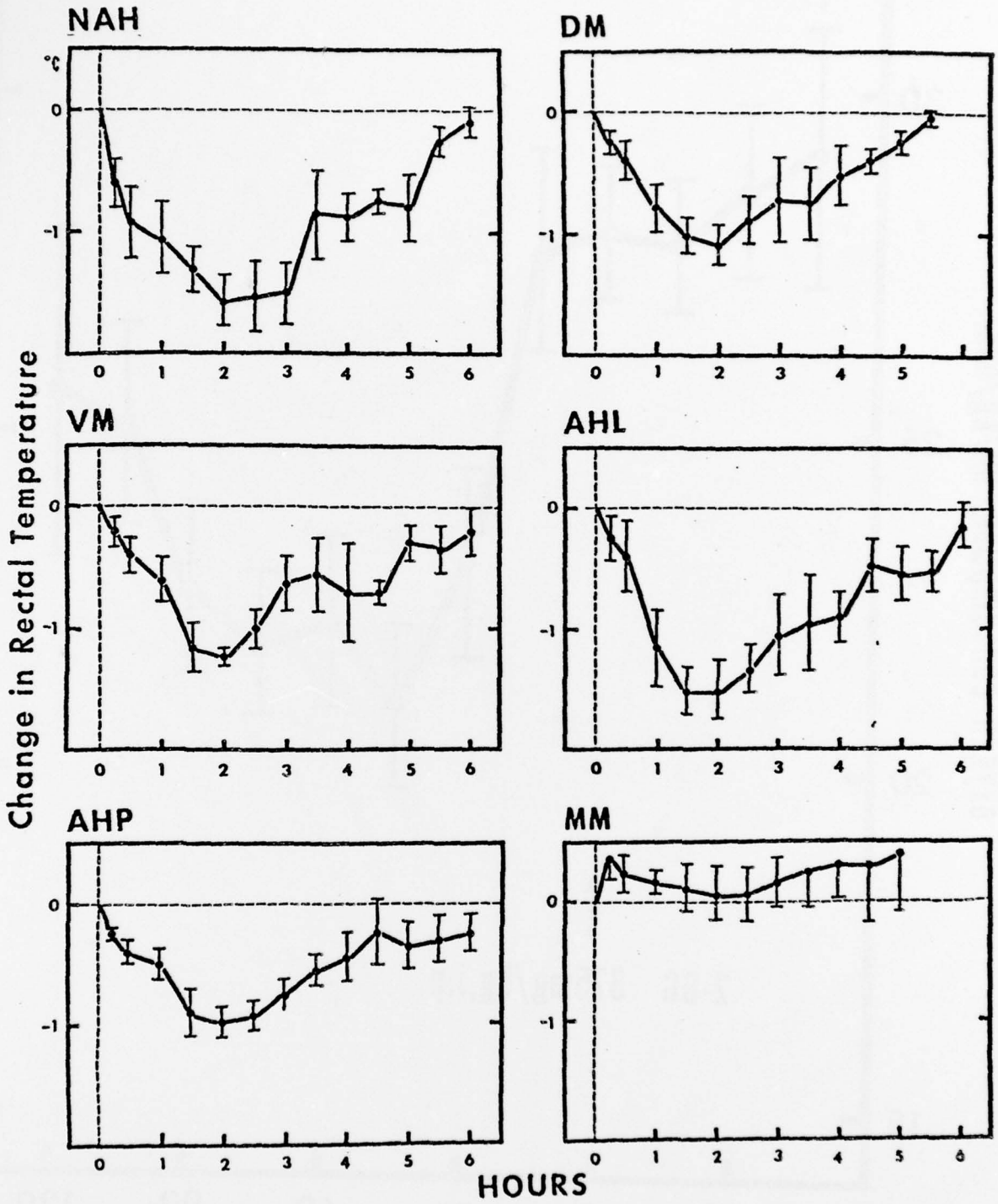
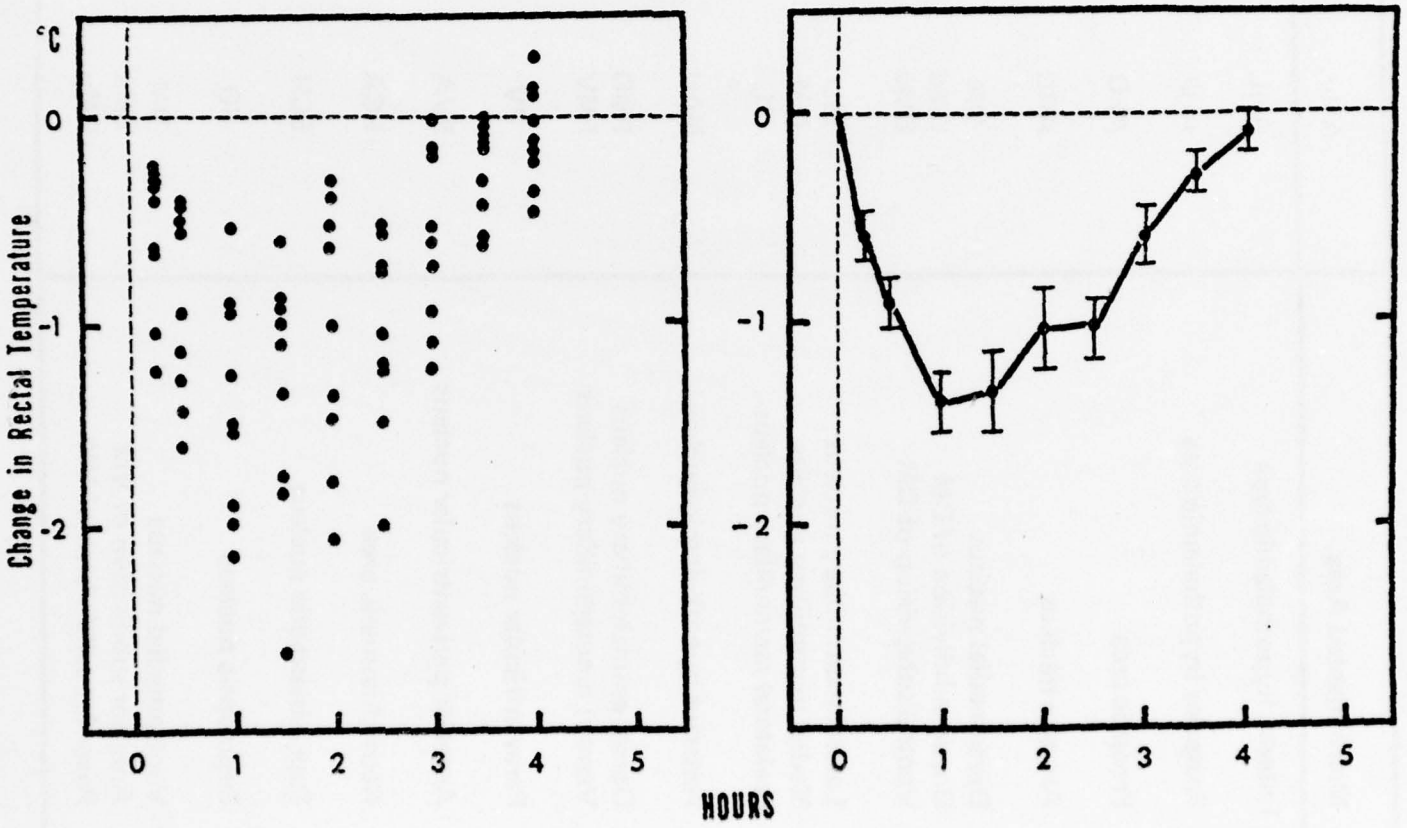

oxygen consumption, ml/kg/min.
30
25
20
15
2-DG 375mg/kg,i.p.
-30
0
30
60
90
120
Time in minutes

Fig 6


NAH
DM
VM
AHL
AHP
MM
°C
0
-1
0 1 2 3 4 5 6
Change in Rectal Temperature
HOURS

2-DG MICROINJECTION: PMV

(20µg/2µl)



F16.7

Table 1

Microinjected Area	Abbr.	Response
Lateral hypothalamic area	AHL	++
Posterior hypothalamic area	AHP	+
Preoptic area	APO	±
Arcuate nucleus	ARC	?
Dorsomedial nucleus	DM	+
Dorsal subdivision of DM	DMd	+
Ventral subdivision of DM	DMv	++
Lateral mammillary nucleus	ML	–
Medial mammillary nucleus	MM	—
Prelateral mammillary nucleus	PL	?
Anterior hypothalamic nucleus	NAH	++
Dorsal premammillary nucleus	PMD	—
Ventral premammillary nucleus	PMV	+++
Paraventricular nucleus	PV	±
Anterior periventricular nucleus	PVA	?
Retrochiasmatic area	RCA	±
Suprachiasmatic nucleus	SCH	±
Supraoptic nucleus	SO	±
Ventromedial nucleus	VM	+
Anterior subdivision of VM	VMa	+
Posterior subdivision of VM	VMp	++

Fig 8

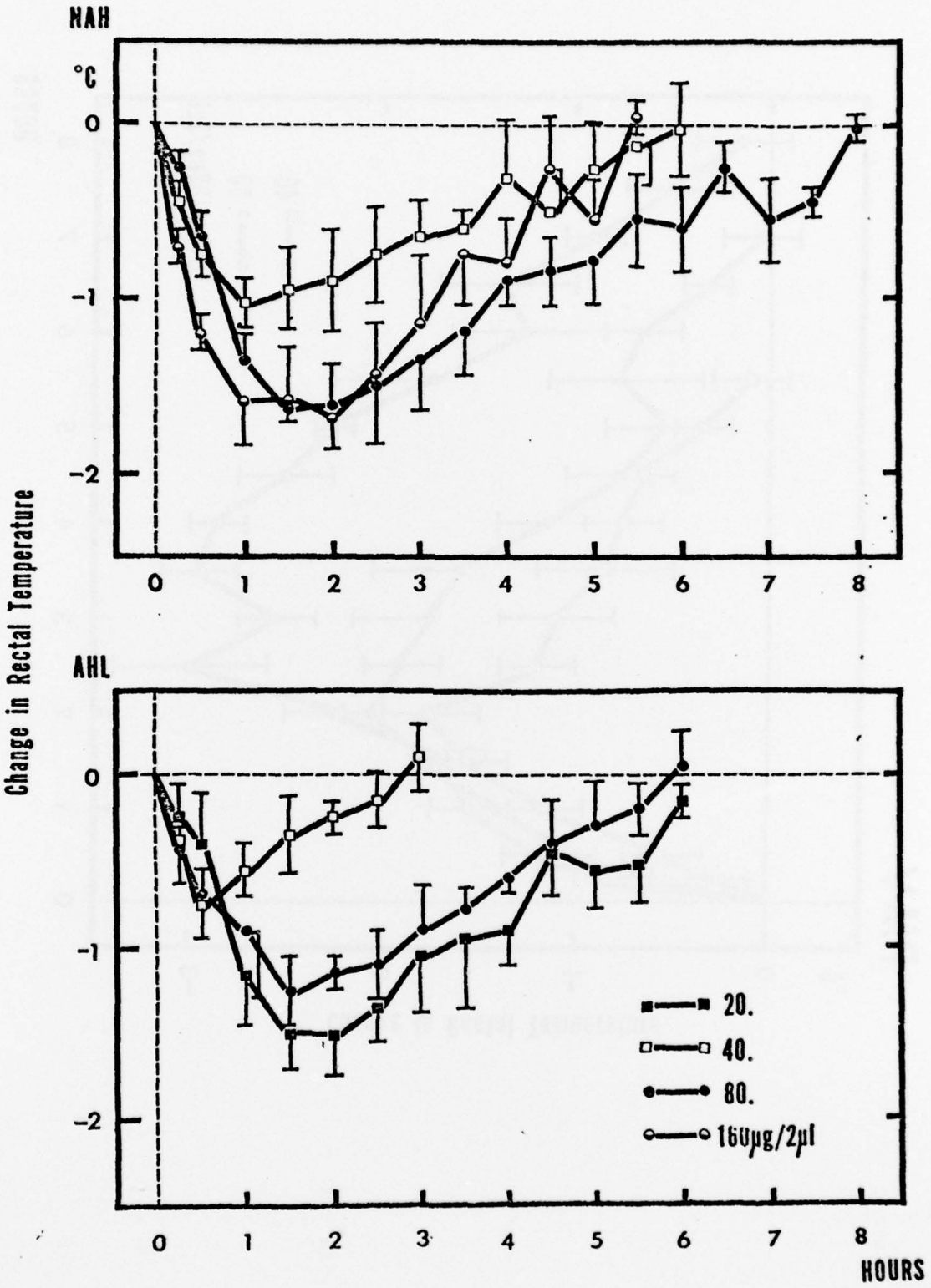
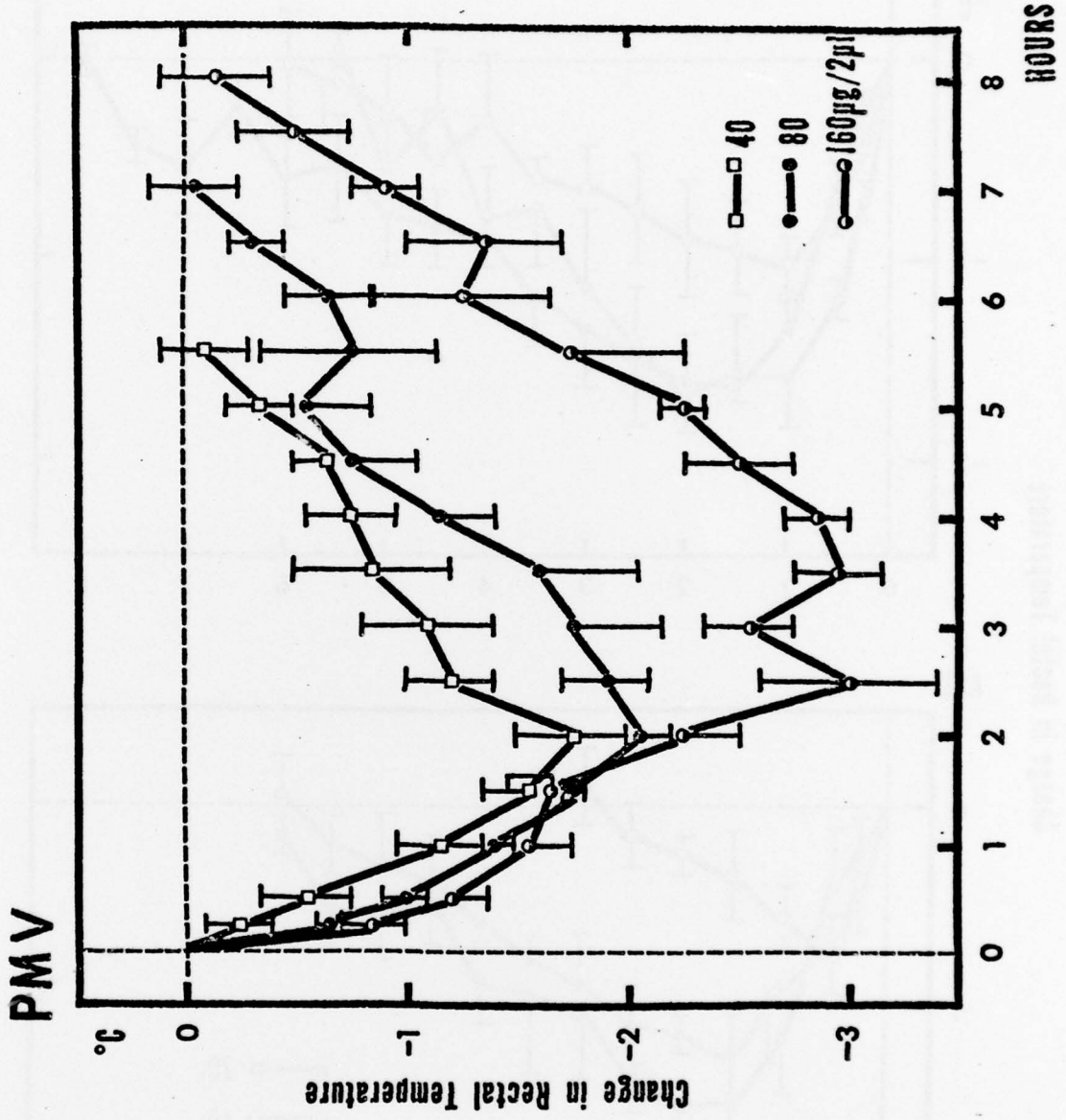

NAH
°C
0
-1
-2
0 1 2 3 4 5 6 7 8
AHL
0
-1
-2
20.
40.
80.
160µg/2µl
0 1 2 3 4 5 6 7 8
HOURS
Change in Rectal Temperature

Fig. 9


PMV
°C
0
-1
-2
-3
Change in Rectal Temperature
0
1
2
3
4
5
6
7
8
HOURS
40
80
160µg/2µl

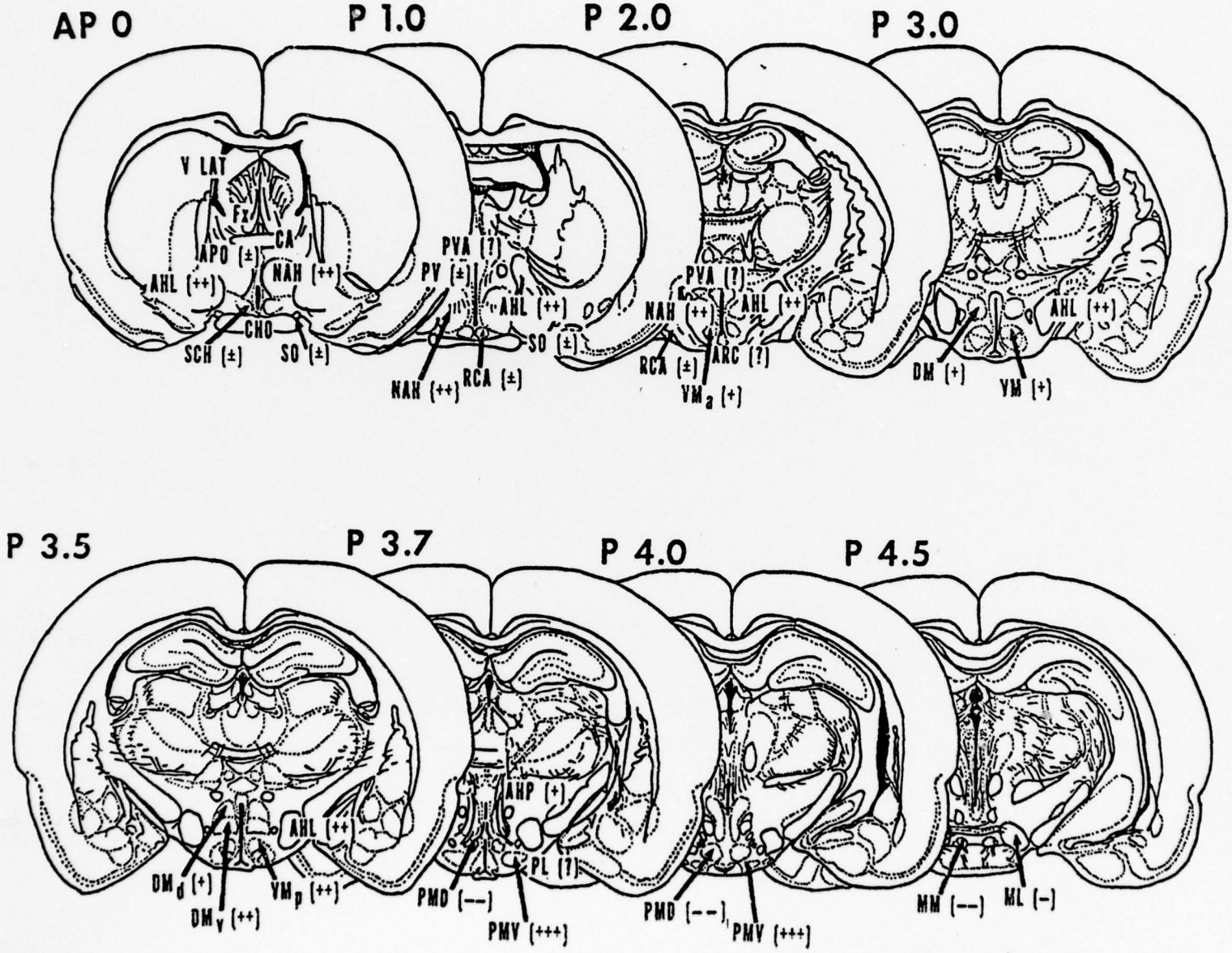

AP 0
P 1.0
P 2.0
P 3.0
V LAT
Fx
CA
APO (±)
AHL (++)
NAH (++)
CHO
SCH (±)
SO (±)
PVA (?)
PV (±)
AHL (++)
NAH (++)
RCA (±)
SO (±)
PVA (?)
NAH (++)
AHL (++)
RCA (±)
ARC (?)
VM$_a$ (+)
AHL (++)
DM (+)
VM (+)
P 3.5
P 3.7
P 4.0
P 4.5
AHL (++)
DM$_d$ (+)
DM$_v$ (++)
VM$_p$ (++)
AHP (+)
PL (?)
PMD (--)
PMV (+++)
PMD (--)
PMV (+++)
MM (--)
ML (-)

FIG. 10

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: M 5/7T (6) (14) USARIEM-M-5/7T
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): Hypothermia Following Injection of 2-Deoxy-D-Glucose into Selected Hypothalamic Sites (10)
5. TYPE OF REPORT & PERIOD COVERED
6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): Takemasa Shiraishi, Milton Mager
8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS: U. S. Army Research Institute of Environmental Medicine, Natick, MA 01760 (16)
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 3A762758A827 T4383106

11. CONTROLLING OFFICE NAME AND ADDRESS: U. S. Army Medical Research & Development Command, Washington, DC 20314 (11)
12. REPORT DATE: Aug 1976 (12)
13. NUMBER OF PAGES: 21 (12) 34p

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): Same
15. SECURITY CLASS. (of this report): Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution of this document is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

NA

18. SUPPLEMENTARY NOTES

NA

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

hypothermia, 2-deoxy-D-glucose, hypothalamus, ventral premammillary nucleus, thermoregulation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

From our previous studies with 2-deoxy-D-glucose (2-DG) an inhibitor of glucose utilization, we postulated that the resultant intracellular glucopenia affects central neuronal pathways involved in the control of peripheral heat production. In this investigation we have delineated these thermoregulatory sites by stereotaxically injecting micro-quantities of 2-DG into the hypothalamus of the rat and monitoring core temperature (T_{re}). After stabilization of T_{re}, 2 ul of 20 ug of 2-DG was injected into 350-400 g rats at 23 ± 1°C.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED 040 850

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Significant decreases in T_{re} were noted for the anterior hypothalamic, ventromedialand dorsomedial nuclei as well as the lateral and posterior hypothalamic areas. With the ventral premammillary nucleus (PMV) mean nadir T_{re} decreases of $-1.1^{o}C$ occurred 1 hour after administration of 2-DG, was significantly depressed after 3 1/2 hours, and returned to basal values after 4 hours. Dose dependant response was observed only for this nucleus. Of a total of 21 sites studied in both the anterior and posterior hypothalamus, the PMV, an area of unknown physiological function, was the most sensitive to glucose deprivation.


78